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Title: Improved Recovery Procedure for Evaluation of Sanitizer Efficacy in Disinfecting Contaminated Cantaloupes

Author(s): B. A. Annous, G.M. Sapers, D.M. Jones, and A. Burke

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Improved Recovery Procedure for Evaluation of Sanitizer Efficacy in Disinfecting Contaminated Cantaloupes

BASSAM A. ANNOUS, GERALD M. SAPERS, DONYEL M. JONES, AND ANGELA BURKE

ABSTRACT: Improved methodology for recovering microbial contaminants from cantaloupe surface is needed. Recovery of bacteria from the entire rind of cantaloupes, obtained with a mechanical peeler, and use of a new method for calculating melon surface area were investigated using melons inoculated with *Salmonella* Poona or *Escherichia coli* NRRL B-766. Growth of *Salmonella* but not *E. coli* was found during post-inoculation storage at 20 °C. The new sampling methodology was equivalent to use of replicate rind plugs, taken at multiple sites on the melon surface, in recovery of both organisms. Recovery was the same by both procedures for dip- and spot-inoculated samples, sanitized or not sanitized, and for post-inoculation holding times up to 72 h. Survival of *Salmonella* on dip- and spot-inoculated cantaloupe surfaces following sanitizer wash treatments was similar.

Keywords: cantaloupe, dip-inoculation, spot-inoculation, recovery, disinfection, microbiological safety

Introduction

Numerous outbreaks of foodborne illness associated with *Salmonella* contamination of cantaloupes (Tamplin 1997; CDC 2002) and the detection of *Salmonella* in surveys of imported and domestic cantaloupes (USFDA 2001, 2003) have focused attention of regulatory agencies and researchers on the problem of melon contamination and disinfection. In studies of melon disinfection methods, investigators typically inoculate the melons with a human pathogen or surrogate and then determine the population of the target organism before and after application of the sanitizing agent or other disinfection treatment. Ideally, the inoculation method should simulate natural contamination and should take into account the site of contamination on the melon surface (for example, stem scar versus equator) and whether contamination would occur preharvest, during harvest, or immediately before packing or fresh-cut processing (Gagliardi and others 2003). Furthermore, the method of recovering attached bacteria from the surface of an inoculated or naturally contaminated melon should provide an accurate estimate of the surviving population.

Beuchat and others (2001) discussed some of the factors that should be considered in developing a protocol for inoculation, recovery, and enumeration of test organisms used in sanitizer efficacy studies. Del Rosario and Beuchat (1995) spot-inoculated whole cantaloupes and watermelons with *Escherichia coli* O157:H7 in 0.1% peptone water (PW), stored the inoculated melons for up to 21 d, and recovered the attached bacteria by excising the inoculated surfaces with a scalpel and stomaching with PW. Their populations were expressed on a surface area basis ($\log_{10}$ colony-forming units [CFU]/cm^2). Park and Beuchat (1999) recovered test organisms from spot-inoculated cantaloupes and honeydew melons after treatment by placing the melons in zip-lock freezer bags with neutralizing broth and hand rubbing. They estimated the population recovered in the neutralizing broth. Gagliardi and others (2003) recovered the native microflora from field samples and from commercially washed cantaloupes and honeydew melons by excising 59-mm-dia rind circles, trimming off the flesh, and blending the rind samples with sterile distilled water in a Waring blender (Waring Products, Torrington Conn., U.S.A.). Barak and others (2003) investigated alternative means of recovering and enumerating surface bacteria from dip-inoculated cantaloupes as part of a study of the efficacy of surface sanitation procedures. They used a washing procedure for recovery of the surviving bacteria.

In previous studies we have recovered bacteria from the surface of inoculated melons by excising replicate (20 or more) rind plugs with a cork borer and homogenizing the plugs with PW (Ukuku and others 2001; Ukuku and Sapers 2001; Ukuku and Fett 2002). These studies indicate the critical importance of the time interval between melon inoculation and disinfection. However, the recovery procedure used is tedious, especially with multiple treatments and sufficient replication of trials, and may miss locations of high bacteria load. Recently, we have used a faster and potentially more accurate recovery method in which the entire melon rind is removed with a mechanical peeler and is then blended with PW (Annous and others 2004). We also have used equations to calculate the melon surface area that take into account the diverse shapes of cantaloupes, which may be approximated by oblate spheroids, spheres, or prolate spheroids. The objective of this study was to compare the 2 methods of recovery using inoculated melons to ensure that the new procedure can be used, irrespective of the interval between inoculation and recovery, and whether large areas of the melon are contaminated simulated by dip-inoculation, or contamination is localized, simulated by spot-inoculation. Additionally, we examined the effect of homogenization on recovery from spot-inoculated samples.

Materials and Methods

Raw material source and inoculation procedure

Fresh unwaxed western cantaloupes (full slip, that is, stem scar smooth and indented with no stem fragments), free of visual defects

were purchased at local food stores or from a distributor and stored at 4 °C for no more than 1 wk before use. Individual cantaloupes were dip or spot-inoculated with *Salmonella* Poona RM 2350 (cantaloupe outbreak isolate) obtained from Dr. William Fett (USDA-ARS-ERRC, Wyndmoor, Pa., U.S.A.) or *E. coli* NRRL B-766 (ATCC 9637; obtained from Dr. L. K. Nakamura, USDA-ARS-NCAUR, Peoria, Ill., U.S.A.), a potential surrogate for *S.* Poona RM 2350 (Eblen and others 2005).

Stock cultures were stored in tryptic soy broth (TSB; BBL/Difco, Sparks, Md., U.S.A.) containing 20% glycerol at −80 °C. Working stocks were maintained on tryptic soy agar (TSA; BBL/Difco) slants containing 0.6% yeast extract (TSAYE), stored at 4 °C for 2 to 4 wk. A loop full of culture from a TSAYE slant was transferred into 10 mL of TSB and allowed to grow for approximately 8 h at 37 °C. This culture was used to inoculate 2 L of the same medium at 0.01% (v/v) level and was allowed to grow with shaking at 90 rpm for approximately 18 h at 37 °C in an incubator shaker (Innova 4230, New Brunswick Scientific, Edison, N.J., U.S.A.). The culture was then centrifuged at $6740 \times g$ for 20 min, washed once with 400 mL sterile distilled water, and resuspended in 4 L of sterile distilled water for the *S.* Poona experiments or 0.1% peptone water (PW; BBL/Difco) for the *E. coli* experiments to give a final cell concentration of 8.7 to 9.1 $\log_{10}$ CFU/mL. The *S.* Poona inoculum was stable in deionized water for at least 24 h at room temperature (RT; about 20 °C) as seen by the recovery of similar microbial cell densities at 0, 4, and 24 h.

Chilled melons (4 °C) were dip-inoculated by full immersion in 4 L of inoculum at RT for 5 min, followed by draining and air-drying at RT for 1 h on absorbent towels in a biosafety cabinet. Alternatively, melons were spot-inoculated by applying 10 μL of the inocula described previously to the center of each of 20 22-mm-dia circles marked on the melon surface with a nontoxic, permanent marking pen (Sharpie, series nr 37000; Sanford, Bellwood, Ill., U.S.A.). At the same time, the inoculum strength was determined by diluting with PW and plating on TSA. The spot-inoculated melons were allowed to dry for 2 h at room temperature in a biosafety cabinet. After air-drying, the inoculated cantaloupes were placed in plastic tubs lined with absorbent paper (spot-inoculated melons positioned to avoid contact between inoculation sites and absorbent paper), covered with aluminum foil, and held at 4 °C or 20 °C for 24, 48, or 72 h before recovery and enumeration.

Response of dip- and spot-inoculated cantaloupe melons to disinfection treatments

Experiments were carried out to determine whether the application of representative disinfection treatments influenced the efficacy of the 2 recovery methods and whether the method of inoculation influenced the response of inoculated cantaloupes to these treatments. Dip- or spot-inoculated cantaloupes with *S.* Poona, stored at RT for 24 h post-inoculation, were placed (one at a time) in a covered stainless-steel basket and submerged for 3 min in a 75-L stainless-steel tank (McMaster Carr, Dayton, N.J., U.S.A.) containing 65 L of 1% hydrogen peroxide (H_2O_2; EKA Chemicals, Marietta, Ga., U.S.A.). The temperature of the 1% H_2O_2 solution was maintained at 20 °C or 60 °C with a 3000-watt electric immersion heater (Cleveland Process Corp., Homestead, Fla., U.S.A.), controlled with a custom-built temperature controller. The melons were then drained and rinsed by submersion in 8 L of cool deionized water with agitation for 30 s. Rind plugs and whole rinds of cantaloupes (6 cantaloupes each) were then analyzed for residual surface populations of *S.* Poona (discussed subsequently).

Recovery of bacteria from inoculated cantaloupes by the rind plug method

Composite samples of 20 rind plugs were taken at random locations on the surface of each dip-inoculated melon with a sterile 20-mm-dia stainless-steel cork borer, and the plugs were trimmed to remove adhering flesh, as described by Sapers and others (2001). Rind plugs were removed from the surface of spot-inoculated melons at 20 of the inoculation sites indicated by the ink circles as described previously. The sets of 20 trimmed plugs from each melon were blended with 75 mL 0.1% PW in a 1250-mL glass jar for 1 min at high speed with a Waring Commercial Blender Model 51BL31 (Waring Products). Blended samples were filtered through a sterile filter bag designed for microbiological examination of particulate suspensions (40-μm pore size; Spiral Biotech, Bethesda, Md., U.S.A.), and the filtrates were serially diluted with sterile PW, as required, and surface-plated on the appropriate growth medium (discussed subsequently).

Recovery of bacteria from inoculated cantaloupes by the whole rind method

A commercial peeler (Peel-All Fruit Peeler, Model CP44, Muro Corp., Tokyo, Japan) was used to recover the whole rind. The stem scar and opposite ends of each inoculated melon were excised with a sterile knife before peeling so that the clamps of the peeler would not contact a contaminated surface. The peeler removed a 1.6 ± 0.3mm-thick layer of rind (measured with an analog 6-inch caliper, accuracy ±0.001 inch, U-09923-28; Cole Palmer Instrument Co., Vernon Hills, Ill., U.S.A.) weighing 80 to 160 g, depending on the size of the melon. The peeler was sanitized after each melon was peeled by spraying the blade assembly, lever, and stage with 70% ethanol for about 20 s using a 16 oz (455 mL) Quorpak chemical sprayer bottle (Fisher Scientific, Pittsburgh, Pa., U.S.A.). The cut ends of each melon and the corresponding peeled rind were weighed and combined in a 1250-mL glass blending container with a volume of 0.1% PW equivalent to 4 times the combined rind weight. The sample was blended for 1 min at high speed with the Waring Commercial Blender, and the homogenate was filtered, diluted, and surface-plated on the appropriate growth medium (discussed subsequently).

Enumeration of bacteria recovered from cantaloupes

Enumeration of *E. coli* and *S.* Poona populations was done using the selective media MacConkey agar (MAC; BBL/Difco) and xylose lysine Tergitol-4 agar (XLT-4; BBL/Difco), respectively. In experiments in which cell injury might be expected, TSA was used as a recovery medium for injured cells of *S.* Poona and *E. coli* recovered from cantaloupes. Recovery medium (TSA) plates were incubated at 37 °C for 2 h to allow injured cells to recover, and then overlayed with the appropriate selective medium. All plates were incubated for 24 h at 37 °C, and resultant colonies were counted manually. Cell densities recovered from the dip-inoculated cantaloupes were reported as $\log_{10}$ CFU/cm², obtained by dividing the population recovered from each whole rind (or rind plug composite) homogenate by the calculated melon surface area (discussed subsequently), whereas populations recovered from spot-inoculated cantaloupes were reported as $\log_{10}$ CFU/spot, obtained by dividing the population recovered from the whole rind homogenate from each melon by the number of inoculated spots (or plugs) represented by the sample.

Calculation of the rind surface area

The surface area represented by composite rind plug samples (S_{plugs}) was calculated from the number of plugs comprising the sample (n) and the plug radius r (1 cm) using the following equation:

$$S_{plugs} = n\pi r^2 = n\pi$$

For the calculation of whole rind surface area, cantaloupes were assumed to be either prolate or oblate spheroids or spheres. Before

inoculation, the length of the stem to blossom end axis and the melon width at its equator were measured with a 50-cm slide caliper (Mantax, Haglöf Sweden AB, Långsele, Sweden). From these measurements, the polar radius "c," equatorial radius "a," and ellipticity "e" were obtained, and the surface area "S" was calculated using one of the following sets of equations for a prolate (c > a) or oblate spheroid (a > c) or sphere (a = c) (Weisstein 1999).

Prolate spheroid:

$$S = 2\pi a^2 + 2\pi \frac{ac}{e} \sin^{-1} e$$

where

$$e = \sqrt{\frac{c^2 - a^2}{c^2}} = \frac{\sqrt{c^2 - a^2}}{c} = \sqrt{1 - \frac{a^2}{c^2}},$$

Oblate spheroid:

$$S = 2\pi a^2 + \pi \frac{c^2}{e} \ln\left(\frac{1+e}{1-e}\right)$$

where

$$e = \sqrt{1 - \frac{c^2}{a^2}},$$

Sphere:

$$S = 4\pi a^2$$

Survival of *E. coli* during homogenization of spot-inoculated rind plugs

To estimate population decreases due to interaction of *E. coli* with rind components released by homogenization, uninoculated rind plugs were homogenized and then combined with the inoculum in the same ratio as was used for rind plugs excised from spot-inoculated melons. These results were compared with estimates of the applied *E. coli* population, calculated from the inoculum population density. Additionally, the populations surviving on spot-inoculated melon surfaces after 1 h drying (which were excised as a rind plug and blended, as described previously) and on spot-inoculated rind plugs after 1 h drying and blending were compared with the applied *E. coli* population.

Statistical analyses

Population reduction data were analyzed for differences in response to treatments by analysis of variance (ANOVA) and the Bonferroni least significant difference (LSD) test to separate means. All statistical analyses were performed with *SAS/STAT* software (SAS Inst. 1989).

Results and Discussion

Recovery of *S.* Poona by the rind plug and whole rind methods

Estimates of the *S.* Poona population on dip-inoculated cantaloupes stored for up to 72 h at 20 °C, obtained by the 2 procedures, were in close agreement (Table 1). The population of *S.* Poona increased by about 2 logs during storage at 20 °C, presumably due to growth on the rind surface (Annous and others 2004).

Because cantaloupes might be naturally contaminated at localized sites on the surface (areas of insect damage, contact with bird droppings, and so forth), which can be simulated by spot-inoculation, we compared sampling by the rind plug and whole rind methods for melons that were spot-inoculated with *S.* Poona (applied to marked locations) to confirm the applicability of the whole rind

Table 1—Comparison of rind plug and whole rind sampling methods for recovery of *Salmonella* Poona RM 2350 from the surface of dip-inoculated cantaloupes[a]

Storage of inoculated melon (h at 20 °C)	*S.* Poona population[b] ($\log_{10}$ CFU/cm²)	
	Plug method[c]	Whole rind method[d]
2	4.7 B	4.3 B
24	6.3 A	6.8 A
48	6.7 A	7.0 A
72	6.9 A	7.0 A

[a]Inoculum (in water) population was 8.7 $\log_{10}$ colony-forming units (CFU)/mL. Xylose lysine Tergitol-4 (XLT-4) agar medium used to enumerate *S.* Poona cell densities.
[b]Mean for 3 melons; means in columns with no letter in common are different (*P* < 0.05) by the Bonferroni least significant difference (LSD) mean separation test. No significant difference between plug and whole rind methods (*P* > 0.05). The standard error of the mean and degrees of freedom are 0.59 and 16, respectively.
[c]Based on total cross-sectional area of 20 rind plugs, each with 20 mm dia; homogenate diluted with 0.1% peptone water (PW).
[d]Based on calculated surface area for spheroid or sphere; whole rind homogenate diluted with 0.1% PW.

method to simulation of this mode of contamination. Recovery results (expressed as $\log_{10}$ CFU/spot) were similar for the 2 methods, even when the melons were held for as long as 72 h at room temperature before sampling (Table 2). As was the case with the dip-inoculated cantaloupes (Table 1), the population of *S.* Poona appeared to increase on the melon surface during the 1st 24 h of storage at 20 °C.

Response of dip- and spot-inoculated cantaloupe melons to disinfection treatments

We suspected that the bacteria on spot-inoculated cantaloupes might be more vulnerable because of looser attachment to the rind surface. If true, the spot-inoculated bacteria might be detached more readily by the sanitizer wash treatments used. Therefore, experiments were carried out to determine whether dip- and spot-inoculated melons as well as the sampling methods used responded differently to representative antimicrobial treatments. Treatment of dip- and spot-inoculated melons with 1% H_2O_2 at 20 °C for 3 min resulted in no significant reduction in *S.* Poona populations (Table 3 and 4, respectively). Similar results were reported by Sapers and Sites (2003) for dip-inoculated cantaloupes with *E. coli* NRRL B-766 that were treated for 15 min in 1% H_2O_2 at 20 °C. Annous and others (2004) reported no change in *S.* Poona populations on dip-inoculated melons after a 3-min wash in tap water at 20 °C. Partial internalization within the netting and/or biofilm formation by *S.* Poona on the surface of the dip- and spot-inoculated cantaloupes may account for the resistance to this 1% H_2O_2 treatment. Treatment by immersion in 1% H_2O_2 at 60 °C resulted in limited but significant (*P* < 0.05) population reductions with both the dip- and spot-inoculated melons. The greater efficacy of the hot 1% H_2O_2 treatment may be the result of heat transfer to bacteria in the netting and/or biofilm and/or greater antimicrobial activity of H_2O_2 at the higher temperature. A surface pasteurization treatment of dip-inoculated cantaloupes with hot water at 75 °C for 3 min resulted in excess of 5 log CFU/cm² reduction in *S.* Poona populations (Annous and others 2004).

Sampling using rind plugs and whole rind methods were not significantly different (*P* > 0.05) for dip-inoculated cantaloupes following sanitizer treatments (Table 3). The rind plug method was significantly lower (*P* < 0.05) than the whole ring method for the spot-inoculated cantaloupes only following 1% H_2O_2 at 60 °C (Table 4). This suggests that the whole rind method, which is more inclusive of the contaminated sites and entails less handling, would be a better sampling method (discussed subsequently).

Table 2—Comparison of rind plug and whole rind sampling methods for recovery of *Salmonella* Poona RM 2350 from the surface of spot-inoculated cantaloupes[a]

Storage of inoculated melon (h at 20 °C)	*S.* Poona population[b] ($\log_{10}$ CFU/spot)	
	Plug method	Whole rind method
2	5.4 B	5.3 B
24	6.6 A	6.2 AB
48	6.1 A	7.4 A
72	6.6 A	7.0 A

[a]Inoculum (in water) population was 8.7 $\log_{10}$ colony-forming units (CFU)/mL. Cantaloupes spot-inoculated using 10 μL of inoculum per spot at 20 locations per melon. Xylose lysine Tergitol-4 (XLT-4) agar medium used to enumerate *S.* Poona cell densities.
[b]Means for 3 melons; means in columns with no letter in common are different ($P < 0.05$) by the Bonferroni least significant difference (LSD) mean separation test. No significant difference between plug and whole rind methods ($P > 0.05$). The root mean square for error and degrees of freedom are 0.92 and 16, respectively.

Table 3—Comparison of rind plug and whole rind sampling methods for recovery of *Salmonella* Poona RM 2350 from the surface of dip-inoculated cantaloupes following 1% hydrogen peroxide wash treatments at 20 °C or 60 °C[a]

Treatment[c]	*S.* Poona population[b] ($\log_{10}$ CFU/cm^2)	
	Plug method[d]	Whole rind method[e]
Control	5.5 A	5.4 A
1% H_2O_2 at 60 °C	4.6 B	4.9 A
1% H_2O_2 at 20 °C	5.2 AB	5.3 A

[a]Inoculum (in water) population was 9.2 $\log_{10}$ colony-forming units (CFU)/mL. Tryptic soy agar (TSA) with xylose lysine Tergitol-4 (XLT-4) overlay agar medium used to enumerate *S.* Poona cell densities.
[b]Mean for 6 melons; means in columns with no letter in common are significantly different ($P < 0.05$) by the Bonferroni least significant difference (LSD) mean separation test. No significant difference between plug and whole rind methods ($P > 0.05$). The standard error of the mean and degrees of freedom are 0.62 and 30, respectively.
[c]Dip-inoculated cantaloupes were stored at room temperature (RT) before washing with 1% hydrogen peroxide at 20 °C or 60 °C for 3 min followed by a 30-s rinse in deionized water.
[d]Based on total cross-sectional area of 20 rind plugs, each with 20 mm dia; homogenate diluted with 0.1% peptone water (PW).
[e]Based on calculated surface area for spheroid or sphere; whole rind homogenate diluted with 0.1% PW.

Recovery of *E. coli* NRRL B-766 from dip-inoculated cantaloupe by the rind plug and whole rind methods

Recovery trials were carried out with *E. coli* NRRL B-766 because of its potential use as a surrogate for *S.* Poona in pilot plant washing trials where a human pathogen could not be used. Studies carried previously by Eblen and others (2005) showed that the 2 organisms had similar attachment and survival characteristics on inoculated apples. Estimates of the *E. coli* population on dip-inoculated cantaloupes, based on the entire rind, were slightly higher ($P < 0.05$) initially and the same after 48 h when compared with estimates based on the pooled rind plugs (Table 5). Growth of *E. coli* on melon surfaces did not occur at 4 °C (Table 5) or at 20 °C (data not shown).

These estimates were obtained using the calculated surface areas of the whole rinds and pooled rind plugs. The whole rind surface area calculations were made conveniently using a spreadsheet format which, upon entry of the length of the polar axis and width at the equator of each individual melon, measured with calipers, yielded the surface area values. The weight of the whole rind, obtained by mechanical peeling as described herein, cannot be used as a basis for the calculation because the correlation between rind surface area and weight was very poor, although significant ($r = 0.41$ for $n = 100$). This is probably a consequence of variability among melons in size, firmness, and rind thickness. The holding time between inoculation and sampling had no effect on the population estimates by the 2 methods.

Effect of sample handling and blending on recovery

Variability in the recovery of bacteria from inoculated cantaloupes may be due to cell death, strong attachment, or entrapment in the melon tissue at various steps during handling. In studying the recovery of *S.* Poona and *E. coli* from the cantaloupe rind surface, we opted to use blending rather than rinsing to separate the target bacteria from the rind tissue. This is because of the difficulty in detaching the applied cells by a washing process if the interval between inoculation and rinsing exceeds the time required for strong attachment, a condition likely to exist with natural contamination and encountered in our previous sanitizer efficacy studies (Ukuku and others 2001; Ukuku and Sapers 2001). In the present study, we have compared the recovery of *E. coli* NRRL B-766 from inoculated rind homogenate, spot-inoculated rind plugs, and spot-inoculated intact melons in which the spot was excised as a plug as described; the quantity of inoculum applied to these samples was approximately the same. These data indicate that most of the bacteria added to a cantaloupe rind homogenate could be recovered and enumerated, suggesting the likely absence of inhibitory substances in the melon rind (Table 6). However, recovery was much less when the inoculum was applied to isolated plugs, which had to be homogenized, or to the cantaloupe surface, which entailed removal, trimming, and homogenization of rind plugs. These large population reductions are probably due to environmental stresses such as dehydration and lack of nutrients on the exposed rind surface, exposure to the high shear and localized heating during homogenization, and entrapment by suspended solids during filtration of homogenates. Although substantial variability in these recovery values can be seen on an arithmetic scale, the impact of such variability on a log scale is much less, thus explaining the relatively low variability seen in population levels ($\log_{10}$ CFU/spot or cm^2) recovered from replicated controls within experiments and between experiments.

The choice between spot- or dip-inoculation and between use of whole rind or rind plugs to estimate microbial populations on the cantaloupe surface will depend on the objectives of the experiment. Knowledge of the influence of different modes of contamination on ease or difficulty of disinfection is needed to develop better means of sanitizing melons. Spot-inoculation can simulate localized contamination by a bird dropping or insect damage. Spot-inoculation would be better than dip-inoculation to characterize specific attachment sites, for example, the stem scar or the melon surface in contact with the ground, where bacteria might be better able to survive postharvest storage. This can be done with replicated rind plugs taken at the delineated location of each inoculated spot on control and treated melons. Where inoculation is intended to simulate contamination of larger portions of the melon surface as might occur from deposition of contaminated dust particles or during spray irrigation with contaminated water, dip-inoculation is a better simulation of the contamination event. However, localized areas where greater population density or survival is suspected (for example, a bruise or area of decay, the ground spot, or stem scar) can be sampled by the rind plug method. If the intent is to determine the efficacy of a washing or sanitizing treatment, an estimate of the overall population reduction is required. Although this can be done by sampling multiple rind plugs from representative locations, sampling the whole rind is equally accurate and more convenient.

Recovery of contaminants from melons naturally contaminated at

Table 4—Comparison of rind plug and whole rind sampling methods for recovery of _Salmonella_ Poona RM 2350 from the surface of spot-inoculated cantaloupes following 1% hydrogen peroxide wash treatments at 20 °C or 60 °C[a]

Treatment[c]	S. Poona population[b] (log$_{10}$ CFU/spot)	
	Plug method[d]	Whole rind method[e]
Control	6.9 A	7.0 A
1% H$_2$O$_2$ at 60 °C	5.3 B	6.1 B
1% H$_2$O$_2$ at 20 °C	6.6 A	6.4A B

[a]Inoculum (in water) population was 10.7 log$_{10}$ colony-forming units (CFU)/mL. Tryptic soy agar (TSA) with xylose lysine Tergitol-4 (XLT-4) overlay agar medium used to enumerate S. Poona cell densities.
[b]Mean for 6 melons; means in columns with no letter in common are different (P < 0.05) by the Bonferroni least significant difference (LSD) mean separation test. No significant difference between plug and whole rind methods (P > 0.05), except for 1% H$_2$O$_2$ at 60 °C treatment. The standard error of the mean and degrees of freedom are 0.41 and 42, respectively.
[c]Spot-inoculated cantaloupes were stored at room temperature (RT) before washing with 1% hydrogen peroxide at 20 °C or 60 °C for 3 min followed by a 30-s rinse in deionized water.
[d]Based on total inoculated spots using 10 µL of inoculum per spot at 20 locations per melon, homogenate diluted with 0.1% peptone water (PW).

Table 5—Comparison of rind plug and whole rind sampling methods for recovery of _Escherichia coli_ NRRL B-766 from the surface of dip-inoculated cantaloupes[a]

Storage of inoculated melon	E. coli population[b] (log$_{10}$ CFU/cm^2)	
	Plug method[c]	Whole rind method[d]
2 h at 20 °C	6.0 B	6.4 A
2 h at 20 °C + 48 h at 4 °C	6.1 A	6.2 A

[a]Inoculum (in 0.1% peptone water [PW]) population was 10.5 ± 0.4 log$_{10}$ colony-forming units (CFU)/mL.
[b]Means for 2 independent trials, each with 3 melons; means in rows with no letter in common are different (P < 0.05) by the Bonferroni least significant difference (LSD) mean separation test. The root mean square for error and degrees of freedom are 0.24 and 19, respectively.
[c]Based on total cross-sectional area of 20 rind plugs, each with 20 mm dia; homogenate diluted with 0.1% PW.
[d]Based on calculated surface area for spheroid or sphere; whole rind homogenate diluted with 0.1% PW.

localized (but unknown) sites cannot be accomplished accurately or efficiently by the plug method because the entire surface would have to be sampled by taking a very large number of plugs in the hope that the contaminated sites would not be missed. Obviously, it would be preferable to use the whole rind method, which would be less labor-intensive and ensure inclusion of the contaminated sites.

Del Rosario and Beuchat (1995) reported an increase in the population of _E. coli_ O157:H7 on the surface of inoculated cantaloupes and watermelons during storage for 4 d at 25 °C, which they attributed to growth from nutrients in the inoculum (PW and TSB), whereas at 5 °C, the bacterial population decreased, similar to our findings. However, in the present study and in a parallel study reported separately (Annous and others 2004), we found growth of _S._ Poona on the melon surface at room temperature, even when the inoculum was suspended in water so that added nutrients were absent. Our observation that _E. coli_ NRRL B-766 did not grow on the surface of inoculated cantaloupe at 20 °C suggests that this organism might not be a suitable surrogate for _S._ Poona.

Barak and others (2003) reported that recovery of _S._ Poona from inoculated cantaloupes was greater when a rinsing procedure was used in contrast to blending the rind and suggested that this result may have been due to release of inhibitory substances from the rind during blending. However, our recovery study showed little or no such inhibition (Table 6). In contrast to our study, Barak and others (2003) used a more dilute inoculum, resulting in lower populations on the inoculated melons, did not store the melons beyond a 1-h drying period before treatment or recovery, and removed the rind by peeling with a kitchen knife, a procedure likely to entail substantially more handling than was required with the electric peeler

in our procedure. In addition, they expressed the recovered populations as log$_{10}$ CFU/mL, a relative value unrelated to the melon surface that fails to take into account the bacterial cells that are not eluted by a rinse and remain attached to the melon surface.

Gagliardi and others (2003) used an excision and blending procedure comparable to our methodology for melons. However, they expressed their results as CFU per gram of rind, which depends on the thickness of the rind circles removed. Our data indicate that this basis, unlike an area basis, is unreliable and that weight-based data cannot be compared with data of other investigators expressed on an area basis.

Conclusions

The whole rind and rind plug methods of recovering attached bacteria from cantaloupe gave similar results for _S._ Poona or _E. coli_, irrespective of the method of inoculation or post-inoculation storage. The poor correlation between the calculated rind surface area and rind weight demonstrates the value of using a surface area basis for expressing microbial population size on melon surfaces. The ability of _S._ Poona but not _E. coli_ NRRL B-766 to grow on the cantaloupe rind surface at 20 °C indicates that the value of the _E. coli_ NRRL B-766 as a surrogate is limited. Greater recovery of _E. coli_ NRRL B-766 from inoculated rind homogenate than from spot-inoculated rind plugs or spot-inoculated intact melon surface after blending is indicative of microbial inactivation during plug removal and blending but not from rind constituents released during blending. Because of its speed and efficiency, the new whole rind procedure is more amenable than the rind plug method for sampling in well-replicated, multi-treatment experiments. The whole rind procedure is now being used routinely in sanitizer efficacy and surface pasteurization studies with cantaloupes conducted in our laboratory.

Table 6—Effects of storage conditions and sample handling on recovery of _Escherichia coli_ NRRL B-766 from spot-inoculated cantaloupe rind

Sample[a]	Storage time (h) at 20 °C	No. of experiments[b]	Applied population (log$_{10}$ CFU/spot)	Recovery (%)
Rind plugs homogenized; then homogenate inoculated	<1	3	10.0 to 10.5	20 to 96
Rind plugs spot-inoculated and then homogenized	1	3	9.0 to 9.4	1.9 to 4.2
Melon surface spot-inoculated, and rind plugs containing spot removed and homogenized	1	4	9.3 to 9.4	0.16 to 0.66

[a]Samples comprise sets of 10 pooled rind plugs or corresponding homogenates. 10 µL of concentrated E. coli 766 inoculum applied to each rind plugs before homogenization or to each designated spot on melon surface before plug removal and homogenization; plug homogenates inoculated in same proportion.
[b]Each experiment represents 2 to 4 independent replicated trials (individual melons or sets of 10 plugs).

Acknowledgments

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